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Red and green *Carcinus*: how different?

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Abstract

Carcinus maenas is, arguably, the most extensively studied intertidal crab in the world and can be found in a variety of habitats, from rocky intertidal, subtidal, in many areas of the world. Because of its range of habitats, it can tolerant a wide range of salinities and other environmental stressors like hypoxia and desiccation. Some individuals go into a prolonged state of intermoult when they reach a certain size, and turn red in colour. This means that *C. maenas* exhibits colour polymorphism, there being a green colour morph and a red colour morph due to different levels of expression in CYP enzymes. These colour morphs have various behavioural, physiological and biochemical differences which result in them occupying different habitats; the green morphs exhibiting tidal migrations up and down the shore, whilst the red morphs stay in the subtidal. Thus although red morphs are better competitors and are more likely to win fights over mates or prey, green morphs are more tolerant to a wide range of salinities and other stressors and so can exploit a wider range of habitats. Recent studies have suggested that green morphs may also be pre-adapted to deal with anthropogenic stress. Most experiments have involved mainly male samples; however, suggesting future research into female *C. maenas* colour morphs could give a wider picture of the differences between colour morphs.

Introduction

The common green shore crab, *Carcinus maenas* (Linnaeus, 1758) is arguably the most widely studied intertidal crab in the world, its commonness in both the shallow sublitoral and intertidal zones (Marine Biological Association, 1957), along with its size and ease of identification making it especially suitable for experimental work (Crothers, 1967). Compared to other intertidal crabs, *C. maenas* can tolerate a much larger range of salinities and other physiological tolerances (Crothers, 1967), as no other species of crab is as readily found in both subtidal and intertidal zones where it is likely to be exposed to fluctuating levels of salinity, hypoxia and desiccation. Although the common designation, "green shore crab" has implications for the colouration and habitat of the species, it has long been established that the colour of individuals and the habitat they occupy is not consistent across the species. Colour polymorphism was initially noted by Crothers (1968) who reported two separate colour morphs of *C. maenas,* and since then a variety of morphological differences between the two colour morphs have been reported. The first and most obvious of these is the colouration of the ventral exoskeleton and limbs which ranges from pale green to dark red (Crothers, 1968; Reid et al*.,* 1989; McGaw et al*.,* 1992). Red morphs are also noted for having a generally larger and thicker carapace (McGaw et al., 1992; Wolf, 1998), more likely to have epibionts attached to their exoskeletons or be infected with a parasite (Crothers, 1968; Wolf, 98), and are also more likely to have a loss of limbs. There are also various behavioural, physiological and biochemical differences reported between to two colour morphs, such as different tidal migration patterns (Crothers, 1968; Dare and Edwards, 1981; Hunter and Naylor, 1993; Warman et al*.,* 1993), different tolerances to environmental stressors like hypoxia (Aldrich, 1986; Taylor et al., 2009) or fluctuating salinities (Reid et al., 1989; McGaw and Naylor 1992a; Lee et al., 2003; Baeta et al., 2005), different tolerances to anthropogenic pollution (Bjerregaard, 1990, 1980; Aagaard and Depledge, 1993; Aagaard, 1996; Styrishave et al.,1999, 2000; Rewitz et al., 2003; Dam et al., 2006, 2008), differing heart rates and locomotor activity in response to stressors (Aagaard, 1996; Styrishave et al*.,* 1999) and different levels of mating success (Styrishave et al., 2004; Wolf, 1998). Despite these obvious differences, many studies that use *C. maenas* take samples without consideration for the differences between different colour morphs, assuming that they are all from the same population.

The aim of the present review is to critically assess the relevant literature that deals with the behavioural, physiological and biochemical differences between the two colour morphs of *C. maenas*, and to suggest potential areas for future research.

Exoskeleton colour and moulting

Crustaceans have a hard exoskeleton which restricts constant growth, resulting in all crustaceans having to moult frequently in order to grow. It has been suggested that the colour change in *C. maenas* is dependant on the duration of time spent in between moulting (Crothers, 1968; Reid et al., 1997; Styrishave et al., 2004), with individuals who have recently moulted being pale green in colour, and those in prolonged inter-moult or terminal anecdysis ranging from orange to dark red (Reid et al., 1997). This is supported by the fact that red morphs generally have more epibionts attached to their exoskeletons (Crothers, 1968; Wolf, 1998), are more likely to have loss of limbs (Crothers, 1968) and that the colouration only occurs in adult individuals who have already reached a large enough size to reproduce (McGaw et al., 1992; Wolf, 1998).

Recent studies on the expression of cytochrome *P*-450 (CYP) enzymes in the different colour morphs of *C. maenas* (Dam et al., 2000; Rewitz et al., 2003; Dam et al., 2008) provide molecular evidence for this theory. A group of moulting hormones (ecdysteroids) are known to be activated and inactivated by cytochrome *P*-450 (CYP) enzymes (Styrishave et al., 2004; Rewitz et al., 2005), and it was demonstrated in an experimental study (Rewitz et al., 2003) that when crabs were injected with ecdysone, (a steroidal hormone that controls moulting) CYP enzyme gene expression was higher in green morph crabs than red morphs. These studies indicate that CYP enzymes are likely to provide a link between growth and the increased tolerance to stressors displayed by green morphs.

It is not clear, however, how much of these findings can relate to female shore crabs, as almost all studies have been conducted with mainly adult males (with the exception of McKnight et al. (2000) and Lee et al. (2003)). It has also previously been reported that some females can keep their red colouration after moulting (Reid et al., 1997), making it hard to determine the reasoning for any differences found, and much of the competitive advantages described below are only relevant to male shore crabs.

The physiological basis for the colour change is currently unknown, although a number of theories have been put forward. Crothers (1968), suggests that some kind of mechanism in the eyestalk to control the red/green balance of the crabs body colour in response to the light intensity of the environment may be present, and more recently it has been suggested that it could be due to photo-denaturation of the integument pigment, astaxanthin, which is known to turn red when denatured (Jencks and Buten, 1964; Lee, 1977; Reid et al., 1997).

Physiological adaptations

Migration Patterns

Previous studies have shown different individuals of *C. maenas* follow different tidal migratory patterns (Crothers, 1968; Dare and Edwards, 1981; Hunter and Naylor, 1993; Warman et al., 1993), some crabs migrating up onto the shore with the tide and some constantly remaining in the subtidal zone. Crothers (1968) found that there when it came to tidal migration in *C. maenas*, there were three sections within a population that individuals could occupy. Section A crabs consisted of a large proportion of juveniles and green colour morphs that spend low tide hidden under cover on the shore, section B crabs contain a mixture of the two colour morphs and spend low tide beneath the tidemarks but migrate up the shore with the flood and back with the ebb, and section C crabs contain mainly red morphs that spend all 24 hours beneath the tidemarks. This behaviour can result in individuals from section A being stranded on the shore when the tide goes out and being exposed to various environmental fluctuations like hypoxia (Aldrich, 1986; Reid and Aldrich, 1988) or varying salinities (Reid et al., 1989; McGaw and Naylor, 1992a; McGaw and Naylor 1992b; Lee et al., 2003). These suggestions correlate with the idea of red colour morphs being much less prominent on the shore, suggesting that they are not as tolerant as the green colour morphs to environmental stressors (Crothers, 1968).

Hypoxia
The hypothesis that red colour morphs were less adapted to deal with hypoxia than green colour morphs has been tested by various people. One of the initial studies that tested the effects of hypoxia on different colour morphs was that of Aldrich (1986). He measured changes in oxygen consumption during exposure to hypoxia in red and green crabs. However, even though there were different compensations made in response to hypoxia depending on the tide, no significant difference was found between the two colour morphs. The notion that different colour morphs would have varied reactions to environmental hypoxia was explored further by Reid and Aldrich (1988), who also measured the oxygen consumption of the two colour morphs in normoxic and hypoxic seawater using a closed respirometry technique. They found that red colour morphs had a poorer ability to compensate for hypoxia than green colour morphs. This was also displayed through them showing the emersion response at greater oxygen tensions than the green crabs, and having greater mortality when exposed to prolonged anoxia.

Osmoregulation
A number of studies have also investigated the differences between osmoregulatory abilities between the two colour morphs. Reid et al. (1989) tested the hypothesis that red colour morphs are less tolerant of fluctuating salinities than green colour morphs through a few different methods. Firstly by analysing haemolymph samples after exposure to different salinities, secondly by investigating salinity preference behaviour, and thirdly by measuring the rate of mortality in hyposalinity. It was found that green morphs were much better osmoregulators in lower salinities, with a high mortality recorded for red morphs kept in hyposalinity. This hypothesis was again tested by McGaw and Naylor (1992a) who also compared salinity preference behaviour between the two colour morphs and found that red colour morphs vacated lower salinities sooner than green morphs, supporting the idea that red morphs are not as tolerant as green morphs to fluctuating salinities. Since all previous studies have concerned male samples, Lee et al. (2003) used a female sample to test mortality rates in low salinities and found that green colour morphs survived significantly longer than red colour morphs, suggesting that female *C. maenas* probably display similar colour related physiological tolerances to male samples. The fact that green morphs are more adapted to cope with lower salinities is also supported by Baeta et al. (2005), who found that the proportion of green morphs increased with increasing distance up from the mouth of a temperate estuary.

Desiccation
Being emersed for extended periods can increase risk of aerial exposure, which can in turn lead to desiccation. The ability of red colour morphs to deal with desiccation has been suggested to be lower than that of green morphs because of the lesser time spent on the shore by them. This was tested by Reid et al. (1989, 1993) who weighed crabs over time to determine water loss. It was found that although red morphs lost water and died somewhat faster than green morphs, there was no significant difference between the colours.

Although there was no significant difference found between red and green colour morphs of *C. maenas* in terms of desiccation, all the other results demonstrate that there are significant physiological differences between the two colour morphs that should be taken into account in any experiment.

Competitive advantages

There has been speculation about which colour morph has a competitive advantage when it comes to conflicts over mates and prey and many studies have been conducted to determine which colour morph dominates in these conflicts. Kaiser et al. (1990) investigated chelal morphometry, prey-size selection and aggressive competition in the two colour morphs of *C. maenas* by measuring the strength of the forces generated and force duration by the crushing chelae with a model mussel fitted with strain gauzes. The results of this study demonstrate that red colour morphs exert a greater proportion of slow chelal contractions, and maintained them for longer than green crabs. It was also found that red colour morphs would dominate over green morphs in disputes about prey, suggesting that the red colour morphs have a competitive advantage over green colour morphs. Taylor et al. (2009) also found that red morphs had significantly heavier and stronger claws than green morphs, and in another experimental study (McGaw et al. 1992) carapaces of both colour morphs were measured and compared to find that red morphs on average had 0.09mm thicker carapaces than green morphs, and that it takes more force to penetrate the carapaces of red morphs. This suggests that the red morphs do have a competitive advantage, and also supports the afore mentioned theory of red morphs being in a prolonged intermoult giving the carapace time to thicken.

Mating Success

Since it has been reported that male shore crabs are known to engage in inter-male conflicts over receptive females (Berrill and Arsenault, 1982), it is likely that mating success will also be affected by the fact that red morphs are aggressively superior to green morphs. Kaiser et al. (1990) reported that there were significantly more red males found in copular or pre-copular from the baited traps, clearly showing that red morph males are significantly more successful in gaining mates compared to green morphs. This was also tested in the laboratory by allowing conflict over a female between red and green morphs to occur and noting which colour morph ended up in copular or pre-copular with the female. The results also showed that red morphs are much more likely to win mates over green morphs.

Wolf (1998) studied a population of shore crabs in the German Wadden Sea to determine whether the theory put forward by Aldrich (1986) about the two colour morphs being two different life phases was valid. The hypothesis was that the green morphs are in an actively growing life phase, channelling their energy into having a short moulting cycle and building body tissue, and the red morphs being in a prolonged intermoult, using energy to raise their metabolic rate whilst maximising their reproductive effort. It was confirmed that red morphs are on average much bigger than green morphs (correlating with findings from McGaw et al., 1992; McKnight et al., 2000), fitting with the idea that red colouration is correlated with sexual maturity and suggesting that this physical advantage over green morphs increases with size, also supporting the two different life strategies.

Anthropogenic stress

The fact that green colour morphs are more tolerant to environmental stressors could mean that they are pre-adapted to deal with anthropogenic stressors (Styrishave et al., 2000). In an experimental study (Styrishave et al., 2000) the effect of cadmium accumulation and dietary status on fatty acid composition in the two colour morphs was compared to determine any differences. It was discovered that starved green morphs accumulated more cadmium in their hepatopancreas than red morphs (either fed or starved), suggesting a physiological advantage over red morphs due to the idea that the ability to accumulate cadmium in the hepatopancreas may have a positive correlation with the physiological condition of the crab (Bjerregaard, 1988; Bjerregaard, 1990). The levels of fatty acids in the two colour morphs were not found to be significantly different, however this is not surprising when you consider how highly energy demanding both moulting and mating are (Styrishave, 2000)

A more recent experimental study (Dam et al., 2006) that tested differences between susceptibility to pyrene between the two morphs and their ability to metabolise it also implies that the two life strategies arise from physiological differences between the colour morphs, and that these differences ultimately affect the different tolerances to pyrene, and other anthropogenic stressors. In the paper by Styrishave et al. (2000) it is also mentioned that green crabs have better survival rates than red crabs when exposed to copper, indicating they are also better adapted to deal with anthropogenic pollution. Krång and Ekerholm (2006) however, found no difference in mating behaviour between colour morphs contrary to the hypothesis that it would negatively affect red morphs more due to them being less tolerant to other anthropogenic stressors.

Conclusion

It is clear from the large amount of data collected on the two different colour morphs of *C. maenas* that there are significant behavioural, physiological and biochemical differences between the red and green morphs. They have been shown to exhibit different life strategies, the green morphs channelling energy into growth, and the red morphs focusing their energy into reproduction. It is either because of, or as a result of this the two morphs have various morphological differences, the larger, stronger red morphs being more adapted for competitive success, and the green morphs being better adapted to deal with fluctuating environmental stressors. This is demonstrated by green morphs being significantly more abundant than red morphs in intertidal areas and in estuaries, and their ability to adapt to fluctuating salinities and varying levels of hypoxia has been demonstrated in several studies where the red morphs have consistently shown higher rates of mortality and general lower tolerances to environmental stressors. The green morph crabs have also been shown to be generally more tolerant to anthropogenic stressors as well (with the exception of in a study by Krång and Ekerholm (2006)), with studies indicating that anthopogenic pollutants such as cadmium and pyrene have more pronounced negative affects on red morphs compared to green.

More recent research has focused on the genetic expression of CYP enzymes which provide a molocular link between the increased tolerance green morphs show in response to stressors. These enzymes have been shown to have involvement with the activation and inactivation of a group of moulting hormones, and have a significantly higher gene expression in green morphs.

The present paper has highlighted significant differences between the colour morphs, meaning that they should always be taken into account when sampling for experiments. If studies are conducted on population samples that have not taken colour polymorphism into account then the results would not be representative of the population. There is also a lack of research conducted into the differences between colour morphs in female *C. maenas,* so it is not possible to conclude whether the differences between the morphs relate to them as much as to males. There is also the fact that red morph females have been reported to keep their red colouration after moulting (Kaiser et al., 1990) which is as yet, unexplained.

No studies as yet have investigated what triggers shore crabs to enter prolonged intermoult apart from the conclusion that it must have some correlation with maturity, due to there being very few red morphs < 30mm. It is also unclear if it is possible for any individual to follow either life strategy, or whether each individual is genetically programmed to follow one main life strategy. Both colour morphs have some advantages and some disadvantages, red morphs winning more competitive disputes and green crabs being more tolerant to stressors. One other interesting point that can also be brought up from the present review is whether green morphs have become more tolerant to environmental stressors, because they are out-competed for prey and mates in the subtidal zone by the red morphs and had to take refuge in marginal habitats, such as estuaries or intertidal areas, or whether they are just better adapted to exploit a wider range of areas.

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